PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2001-245677

(43)Date of publication of application : 11.09.2001

(51)Int.Cl. C12N 15/09
C12M 1/00
C12Q 1/68
G01N 33/53
G01N 33/566
G01N 33/569
//(C12Q 1/68
C12R 1:42)
(C12Q 1/68
C12R 1:01)

(21)Application number : 2000-398087

(22)Date of filing : 27.12.2000

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(30)Priority

Priority number : 11368920 Priority date : 27.12.1999 Priority country : JP

(54) NUCLEIC ACID FOR ASSAYING BACTERIA BELONGING TO THE GENUS SHIGELLA OR SALMONELLA AND METHOD FOR DETECTING THE BACTERIA

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a means for discriminating Shigella flexneri, Shigella boydii, Shigella sonnei, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium, Salmonella chester, Salmonella enteritidis and Salmonella oranienburg by a gene test to assay.

SOLUTION: This means for discriminately assaying the above nine kinds of bacteria is provided by using a section which consists of a base sequence in the specific domain of gyrase β gene(gyr β) consisting of the structural gene of the β-subunit in these bacterial enzyme topoisomerase II and contains the different base sequence due to each bacterial species as a primer or a probe.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

CLAIMS

[Claim(s)]
[Claim 1] The nucleic acid for shigella FUREKUSUNERI measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 1 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 2 thru/or 9, or its complementary strand all over one of response fields at least.
[Claim 2] The nucleic acid for shigella BOIDI measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 2 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 1 or 3 thru/or 9, or its complementary strand all over one of response fields at least.
[Claim 3] The nucleic acid for shigella ZONEI measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 3 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 1, 2 or 4 thru/or 9, or its complementary strand all over one of response fields at least.
[Claim 4] The nucleic acid for Salmonella typhi measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 4 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 1 thru/or 3 or 5 thru/or 9, or its complementary strand all over one of response fields at least.
[Claim 5] The nucleic acid for Salmonella-paratyphi-A measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 5 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 1 thru/or 4 or 6 thru/or 9, or its complementary strand all over one of response fields at least.

[Claim 6] The nucleic acid for Salmonella typhimurium measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 6 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 1 thru/or 5 or 7 thru/or 9, or its complementary strand all over one of response fields at least.

[Claim 7] The nucleic acid for the Salmonella Chester measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 7 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 1 thru/or 6, 8, or 9, or its complementary strand all over one of response fields at least.
[Claim 8] The nucleic acid for the Salmonella enteritidis measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 8 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 1 thru/or 7, or 9, or its complementary strand all over one of response fields at least.
[Claim 9] The nucleic acid for the Salmonella ORANIEMBAGU measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which is the complementary strand of the nucleic acid which has the base sequence shown in the array number 9 of an array table, or this nucleic acid, or its fragment, and has the base sequence shown in the array number 1 thru/or 8, or its complementary strand all over one of response fields at least.
[Claim 10] A nucleic acid given in claim 1 which consists of the fragment which has a different base from the base in said fragment of the nucleic acid which has the base sequence shown in the array number 20 of an array table thru/or 29, or its complementary strand all over one of response fields at least thru/or any 1 term of 9.
[Claim 11] A nucleic acid given in any 1 term of claims 1-10 whose numbers of bases are 8-50.

[Claim 12] A nucleic acid given in claim 1 which is a primer for nucleic-acid magnification thru/or any 1 term of 11.
[Claim 13] The nucleic acid according to claim 12 with which it is a forward side primer and said different base is located in the three-dash terminal of a nucleic acid.
[Claim 14] A nucleic acid given in claim 1 used as a nucleic acid probe thru/or any 1 term of 11.
[Claim 15] Two or more nucleic-acid chips for bacteria detection chosen from the group which consists of shigella FUREKUSUNERI which immobilizes two or more nucleic acid probes which consist of two or more nucleic acids indicated by claim 1 thru/or two or more claims of 9 on a base material, and changes, shigella BOIDI, shigella ZONEI, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium, Salmonella Chester, Salmonella enteritidis, and Salmonella ORANIEMBAGU.
[Claim 16] The nucleic-acid chip according to claim 15 which immobilized on the base material by using two or more nucleic acids as said nucleic acid probe among the nucleic acids which have the base sequence shown in the array number 30 thru/or 39.
[Claim 17] Shigella FUREKUSUNERI which immobilizes at least nine kinds of nucleic acids indicated by claim 1 thru/or all the claims of 9, respectively on a base material, and changes, shigella BOIDI, shigella ZONEI, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium, Salmonella Chester, Salmonella enteritidis, and the nucleic-acid chip for the Salmonella ORANIEMBAGU detection.
[Claim 18] The nucleic-acid chip according to claim 17 which immobilized on the base material by using as said nucleic acid probe the nucleic acid which has the base sequence shown in the array number 30 thru/or 39.
[Claim 19] A nucleic-acid chip given in claim 15 whose numbers of bases of a nucleic acid probe are 8 thru/or 25 thru/or any 1 term of 18.
[Claim 20] The detection approach of shigella FUREKUSUNERI which is the nucleic acid which has the base sequence shown in the array number 1 of an array table, or its fragment, amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 2 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least.
[Claim 21] The detection approach of shigella BOIDI which is the nucleic acid which has the base sequence shown in the array number 2 of an array table, or its fragment, amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 or 3 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least.
[Claim 22] The detection approach of shigella ZONEI which is the nucleic acid which has the base sequence shown in the array number 3 of an array table, or its fragment, amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1, 2 or 4 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least.
[Claim 23] The detection approach of Salmonella typhi which is the nucleic acid which has the base sequence shown in the array number 4 of an array table, or its fragment, amplifies at least the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 3 or 5 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment.
[Claim 24] The detection approach of Salmonella paratyphi A which is the nucleic acid which has the base sequence shown in the array number 5 of an array table, or its fragment, amplifies at least the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 4 or 6 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment.
[Claim 25] The detection approach of Salmonella PARACHIFIMURIUMU which is the nucleic acid which has the base sequence shown in the array number 6 of an array table, or its fragment, amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 5 or 7 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least.
[Claim 26] The detection approach of Salmonella Chester which is the nucleic acid which has the

base sequence shown in the array number 7 of an array table, or its fragment, amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 6, 8, or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least.
[Claim 27] The detection approach of Salmonella enteritidis which is the nucleic acid which has the base sequence shown in the array number 8 of an array table, or its fragment, amplifies at least the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 7, or 9 from the base in this fragment, and includes determining the base sequence of this fragment.
[Claim 28] The Salmonella ORANIEMBAGU detection approach which is the nucleic acid which has the base sequence shown in the array number 9 of an array table, or its fragment, amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 8 from the base in this fragment, and includes determining the base sequence of this fragment at least.
[Claim 29] The primer for nucleic-acid magnification which consists of the nucleic acid which is used for magnification of the nucleic acid indicated by claim 20 thru/or any 1 term of 28, and has a base sequence the same as that of one distal region of the base sequences of the nucleic-acid field which should be amplified, or complementary.

[Translation done.]

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the nucleic acid for measurement and the detection approach of a shigella group or the Salmonella bacteria.
[0002]
[Description of the Prior Art] In shigella group bacteria lists, such as shigella FUREKUSUNERI (Shigella flexneri), shigella BOIDI (Shigella boydii), and shigella ZONEI (Shigella sonnei), Salmonella typhi (Salmonella typhi), Salmonella paratyphi A (Salmonella paratyphiA), Salmonella typhimurium (Salmonella typhimurium), Salmonella Chester (Salmonella chester), The Salmonella bacteria, such as Salmonella enteritidis (Salmonellaenteritidis) and Salmonella ORANIEMBAGU (Salmonella oranienburg) It is important on medical care and it necessary to identify these bacilli as an infectious disease or a disease germ of food poisoning, for the diagnosis, a therapy, or prevention.

[0003] Conventionally, identification of a bacillus cultivates a bacillus and is mainly performed by conducting biochemical inspection of utilization nature inspection of sugar etc. However, by this approach, since culture of a bacillus takes time amount, it has the fault that neither diagnosis of an infectious disease or food poisoning, therapy nor prevention can be performed promptly.
[0004] On the other hand, genetic screening which identifies a bacillus is also conducted based on the gene sequence of a bacillus. Since genetic screening does not need culture of a bacillus, it has the advantage that it can inspect promptly. By genetic screening, in order to identify the bacilli of a close relationship, the difference of the base sequence of 16S ribosomal RNA (rRNA) is used.
[0005] Moreover, the approach the difference of the base sequence of the gyrase beta gene (gyrB) which is a structural gene of beta subunit of the enzyme topoisomerase II detects the Bacteroides bacteria, Mycobacterium bacteria, KICHINOFAGA group bacteria, the Flavobacterium bacteria, Cytophaga group bacteria, SHINEKOKOKKASU group bacteria, the Caulobacter group bacteria, the Pseudomonas bacteria, etc. is indicated by JP,11-169175,A. However, detection of shigella group bacteria and the Salmonella bacteria is not indicated at all by JP,11-169175,A.
[0006]
[Problem(s) to be Solved by the Invention] Each of three kinds of above-mentioned shigella group bacteria and six kinds of Salmonella bacteria belongs to Enterobacteriaceae, and since it is extremely similar and the base sequence of 16SrRNA is also extremely similar, the gene sequence cannot identify the nine above-mentioned sorts of bacteria by the conventional approach based on the base sequence of 16SrRNA, and cannot be identified.
[0007] Therefore, the object of this invention is offering a means identifying and measuring shigella FUREKUSUNERI, shigella BOIDI, shigella ZONEI, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium, Salmonella Chester, Salmonella enteritidis, and Salmonella ORANIEMBAGU by genetic screening.
[0008]
[Problem(s) to be Solved by the Invention] Wholeheartedly, as a result of research, invention-in-this-application persons hit on an idea of measuring the nine above-mentioned kinds of bacilli for whether the header differs that the base sequences of a part with the gyrase beta gene (gyrB) which is a structural gene of beta subunit of the enzyme topoisomerase II of these bacteria differ in extent which can identify each strain from the base of which part in discernment by the header and it, and completed this invention.
[0009] That is, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 1 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for shigella FUREKUSUNERI measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 2 thru/or 9, or its complementary strand all over one of response fields at least. Moreover, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 2 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for shigella BOIDI measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 1 or 3 thru/or 9, or its complementary strand all over one

of response fields at least. Moreover, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 3 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for shigella ZONEI measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 1, 2 or 4 thru/or 9, or its complementary strand all over one of response fields at least. Moreover, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 4 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for Salmonella typhi measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 1 thru/or 3 or 5 thru/or 9, or its complementary strand all over one of response fields at least. Furthermore, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 5 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for Salmonella-paratyphi-A measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 1 thru/or 4 or 6 thru/or 9, or its complementary strand all over one of response fields at least. Furthermore, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 6 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for Salmonella typhimurium measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 1 thru/or 5 or 7 thru/or 9, or its complementary strand all over one of response fields at least. Furthermore, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 7 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for the Salmonella Chester measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 1 thru/or 6, 8, or 9, or its complementary strand all over one of response fields at least. Furthermore, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 8 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for the Salmonella enteritidis measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 1 thru/or 7, or 9, or its complementary strand all over one of response fields at least. Furthermore, this invention is the complementary strand of the nucleic acid which has the base sequence shown in the array number 9 of an array table, or this nucleic acid, or its fragment, and offers the nucleic acid for the Salmonella ORANIEMBAGU measurement which consists of the fragment which has a different base from the base in this fragment of the nucleic acid which has the base sequence shown in the array number 1 thru/or 8, or its complementary strand all over one of response fields at least.

[0010] Furthermore, this invention is the nucleic acid which has the base sequence shown in the array number 1 of an array table, or its fragment, and offers the detection approach of shigella FUREKUSUNERI which amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 2 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least. Moreover, this invention is the nucleic acid which has the base sequence shown in the array number 2 of an array table, or its fragment, and offers the detection approach of shigella BOIDI which amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 or 3 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least. Moreover, this invention is the nucleic acid which has the base sequence shown in the array number 3 of an array table, or its fragment, and offers the detection approach of shigella ZONEI which amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1, 2 or 4 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least. Furthermore, this invention is the nucleic acid which has the base sequence shown in the array number 4 of an array table, or its fragment, and offers the detection approach of Salmonella typhi which amplifies the fragment which

has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 3 or 5 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least. Furthermore, this invention is the nucleic acid which has the base sequence shown in the array number 5 of an array table, or its fragment, and offers the detection approach of Salmonella paratyphi A which amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 4 or 6 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least. Furthermore, this invention is the nucleic acid which has the base sequence shown in the array number 6 of an array table, or its fragment, and offers the detection approach of Salmonella PARACHIFIMURIUMU which amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 5 or 7 thru/or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least. Furthermore, this invention is the nucleic acid which has the base sequence shown in the array number 7 of an array table, or its fragment, and offers the detection approach of Salmonella Chester which amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 6, 8, or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least. Furthermore, this invention is the nucleic acid which has the base sequence shown in the array number 8 of an array table, or its fragment, and offers the detection approach of Salmonella enteritidis which amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 7, or 9 from the base in this fragment, and includes determining the base sequence of this fragment at least. Furthermore, this invention is the nucleic acid which has the base sequence shown in the array number 9 of an array table, or its fragment, and offers the Salmonella ORANIEMBAGU detection approach which amplifies the fragment which has a different base all over one of response fields of the nucleic acid which has the base sequence shown in the array number 1 thru/or 8 from the base in this fragment, and includes determining the base sequence of this fragment at least. Furthermore, the primer for nucleic-acid magnification which consists of the nucleic acid which this invention is used for magnification of the nucleic acid in the approach of these this inventions, and has a base sequence the same as that of one distal region of the base sequences of the nucleic-acid field which should be amplified, or complementary is offered.

[0011]

[Embodiment of the Invention] The base sequence of the specific field of shigella FUREKUSUNERI, shigella BOIDI, shigella ZONEI, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium, Salmonella Chester, Salmonella enteritidis, and the gyrB gene of Salmonella ORANIEMBAGU is shown in the array numbers 1-9 of an array table, respectively. Moreover, drawing 1 -3 are aligned and the base sequence shown in the array numbers 1-9 is shown in them (although the base sequence shown in the array numbers 1-9 and the base sequence shown in drawing 1 -3 are the same in addition, priority is given to drawing 1 -3 when there should be a difference). In drawing 1 -3, only all the base sequences of shigella FUREKUSUNERI are shown, about the base sequence of other bacteria, a dot shows the same base as the base sequence of shigella FUREKUSUNERI, and only the part of a different base is shown by the alphabet. Therefore, if drawing 1 -3 are seen, it is quite obvious the base of which part of which bacillus is [how] different. This invention makes it the principle to identify and measure nine kinds of above-mentioned bacilli by the difference of the base sequence shown in the array numbers 1-9 and drawing 1 -3.

[0012] The nucleic acid for bacillus detection of this invention includes the primer for nucleic-acid magnification, and a probe. First, the primer for nucleic-acid magnification is explained. the nucleic-acid fragment which has the base sequence of the gyrB gene of the bacillus the primer for measuring each bacillus is indicated to be to the array numbers 1-9, or the same base sequence as a part of the complementary strand -- it consists of a DNA fragment preferably. For example, if it is a primer for shigella FUREKUSUNERI detection, it has the base sequence shown in the array number 1, or the same base sequence as a part of the complementary strand. Furthermore, a primer has at least one different base from the base in any one response field at least of eight kinds of other bacilli. As for the same fragment, a paraphrase does not use all the base sequences of the response field of nine

kinds of bacilli as a primer here. In addition, in this description, as shown in drawing 1 thru/or drawing 3 or below-mentioned drawing 4 thru/or drawing 11 , when aligning the base sequence of a gyrB gene, a field with a certain array and the field of other arrays located in the same location of a lengthwise direction are meant as a "response field." since the primer for measurement of each bacillus is efficiently hybridized since the base sequence is completely complementary about the bacillus, and a primer cannot hybridize it efficiently about the bacillus which has a different base (mismatch base) all over a response field, it almost has magnification -- it is -- it can avoid occurring at all Therefore, it can know whether the bacillus contained in the group in which a specimen includes the bacillus or its bacillus is included by using such a primer. In addition, the primer may include two or more mismatch parts. In order to secure that the gene of other bacilli which have a mismatch base is not amplified, a primer is a forward side primer and it is desirable to set up a primer so that the three-dash terminal may serve as a mismatch part. If it does in this way, even if it can hybridize with a primer other bacilli which have a mismatch base, the three-dash terminal of a primer is un-complementary, since involution cannot be carried out, a chain will not develop any more, therefore magnification will not break out.

[0013] The field set up as a primer is set up so that it may have any one response field and the mismatch base of eight kinds of other bacilli at least. This can be easily performed with reference to drawing 1 -3. For example, in setting up the primer which discriminates shigella FUREKUSUNERI from eight kinds of bacilli other than this, and measures it, the 87th T (it indicates like "87nt" hereafter) uses the forward side primer used as a three-dash terminal. Although 87nt of shigella FUREKUSUNERI is T so that clearly from drawing 1 , with eight kinds of other bacilli, all corresponding bases are C. Therefore, if a three-dash terminal amplifies using the forward side primer used as T which is 87nt, only when shigella FUREKUSUNERI is contained in a specimen, magnification breaks out, and when other bacilli are contained, magnification will not break out. Therefore, other than this, the bacillus contained in a specimen can identify or in shigella flexible SHINERI by using this primer. In addition, the above-mentioned discernment can be ensured by performing magnification actuation similarly in this case using the primer whose three-dash terminal is C which is the response base of eight kinds of other bacilli, and checking whether magnification breaks out.

[0014] Thus, as a primer in which only the bacillus to measure contains the base from which other all and response bases of eight kinds of bacilli differ, in for shigella FUREKUSUNERI measurement, the primer containing 354nt A, 399nt A, 441nt T, 567nt T, 759nt G, 786nt T, and 888nt T can be mentioned so that clearly [the 87nt above-mentioned others] from drawing 1 .

[0015] The primer of this invention is not limited to the primer in which only the bacillus measured as mentioned above contains the base from which other all and response bases of eight kinds of bacilli differ, and also includes the primer containing the base of eight kinds of other bacilli from which any one response base differs at least. For example, in for shigella FUREKUSUNERI measurement, the forward side primer containing 39nt T which makes this T a three-dash terminal preferably can be mentioned. As for five sorts of Salmonella, the response base is G although the response base of two sorts of everything [base / this] but a shigella group is T like both FUREKUSUNERI. Therefore, if such a primer is used, in the case of either of three sorts of a shigella group, magnification will break out [the bacillus in a specimen], and, in the case of either of five sorts of Salmonella, magnification will not break out. Therefore, the bacillus contained in a specimen can perform five sorts of discernment of three sorts of a shigella group, and Salmonella by using such a primer. By authorizing using other suitable primers similarly, when each primer is used, based on the pattern of whether magnification breaks out, a bacillus can be identified easily (it is specified as a single kind). thus, a group which includes shigella FUREKUSUNERI in "the nucleic acid for shigella FUREKUSUNERI measurement" in this description -- it is defined as that by which the nucleic acid used in order to measure a seed is also included.

[0016] In measurement of the bacillus using the above-mentioned primer of this invention, the primer of another side can be set as the same field in nine sorts of all bacilli that what is necessary is just the primer which has the mismatch part of the primer of a couple used for magnification which only either described above at least. As mentioned above, since the forward side primer which makes a mismatch part a three-dash terminal is desirable as a primer of this invention, it is desirable to set a

reverse side primer as nine sorts of common areas, using such a primer as a forward side primer.
[0017] In addition, the nucleic-acid amplifying method itself, such as PCR, is common knowledge in this field, and since the kit and equipment for it are also marketed, if a primer is set up, it can perform nucleic-acid magnification easily by using the gene of the bacillus in a specimen as mold. Moreover, it is also possible by performing the so-called real-time detection PCR using a quencher fluorochrome and a reporter fluorochrome to carry out the quantum of the cell mass in a specimen. Therefore, in this description, both detection and quantum are included with "measurement." In addition, since the kit for the real-time detection PCR is also marketed, it can carry out easily.
[0018] Although it is also good to perform nucleic-acid magnification once about each measurement, measurement can still be ensured by performing the so-called nested nucleic-acid magnification which amplifies a big field by the 1st time, and amplifies the field in the magnification field by the 2nd time. In this case, the 1st primer for magnification can also be set to the outside of the array shown in the array numbers 1-9 and drawing 1 -3. An example of the primer array set as the array number 10 by the downstream (3' side) in an example of the array of the primer set as the upstream (5' side) of the array shown in the array numbers 1-9 and drawing 1 -3 is shown in the array number 11. A fragment including all the fields of the array which sets a primer as these fields and is first shown in the array numbers 1-9 and drawing 1 -3 can be amplified, and, subsequently to the 2nd time, magnification using the primer of above-mentioned this invention can also be performed.
[0019] In addition, although especially the size of a primer is not limited, 15 - 50 base extent is desirable, and 15 - 30 base extent is still more desirable. Moreover, a primer can be easily prepared by chemosynthesis.
[0020] This invention also offers the nucleic acid used as the above-mentioned primer, and the nucleic acid probe which gives an indicator to DNA and grows into it preferably. Since the mismatch part is included, this probe does not hybridize the nucleic acid which has a mismatch base, although the kind which should be measured, and the nucleic acid which has the same base sequence are hybridized. therefore, a group which includes the kind or this kind which should be measured by using such a probe like the case of a primer -- a seed and the other kind are discriminable. The explanation about a mismatch part is the same as that of the case of the above-mentioned primer. But it is not necessarily desirable that a three-dash terminal is especially a mismatch part unlike a primer. Rather, it is desirable to have at least one mismatch part in the range of 3 / 10 - 7/10 with the number of bases from one edge of a nucleic-acid fragment preferably near the center of a nucleic acid probe. Moreover, it can also investigate whether as the probe which does not attach an indicator is immobilized to base materials, such as film, and the indicator of the magnification object of a specimen gene is carried out, a specimen gene amplification object is combined with the film through a probe. Thus, what investigates whether it hybridizes with a specimen gene also calls a "probe" the nucleic acid which does not attach an indicator in this description. That is, the above-mentioned primer will be used as a nucleic acid probe as it is in this case. Immobilization on the base material of a nucleic-acid fragment can be performed by the well-known approach using commercial equipment.
[0021] Although not limited, since the homology between each bacillus is high when too long, even if the specimen contains the mismatch base, it comes to hybridize especially the size of a nucleic acid probe. Therefore, the size of a probe has desirable 8 - 25 base extent, and its eight to 20 base is still more desirable. As an indicator, the well-known indicator used for the conventional nucleic acid probe can be used, for example, a radiolabel, fluorescent labeling, enzyme labeling, a biotin indicator, etc. can be mentioned. Moreover, a probe can be used like the conventional probe.
[0022] Each above-mentioned shigella group of this invention or the above-mentioned nucleic acid for the Salmonella bacteria measurement It adds to nine sorts of above-mentioned bacteria. Further Salmonella paratyphi B (Salmonella paratyphiB), An Escherichia coli O-157 stock (Escherichia coli O-157), Yersinia en TEROKORITIKA (Yersinia enterocolitica), Yersinia RUKERI (Yersinia ruckeri), Enterobacter cloacae (Enterobacter cloacae), Enterobacter aerogenes (Enterobacter aerogenes), Vibrio alginolyticus (Vibrio alginolyticus), A vibrion comp berry (Vibrio campbellii), At least one or more sorts of the bacteria chosen from ten sorts of groups which consist of vibrion diazo trophy dregs (Vibrio diazotrophicus) and vibrion GAZOGENESU (Vibrio gazogenes), It is preferably desirable that it is what can identify seven or more sorts still more preferably, and can

measure the nine above-mentioned sorts of shigella groups or the Salmonella bacteria five or more sorts. Like nine sorts of above-mentioned shigella groups or the above-mentioned Salmonella bacteria, since ten sorts of these bacteria are important as a cause bacillus of food poisoning, for the object which specifies the cause bacillus of food poisoning, it is advantageous that it discriminates from these ten sorts of bacteria, and the nine above-mentioned sorts of shigella groups or the Salmonella bacteria can be identified. The base sequence of the gyrB gene of ten sorts of these bacteria is shown in the array number 20 thru/or 29, respectively. Moreover, that where these arrays were aligned with the gyrB array of the nine above-mentioned sorts of shigella groups or the Salmonella bacteria is shown in drawing 4 thru/or drawing 11 . In addition, in drawing 4 thru/or drawing 11 , like drawing 1 thru/or drawing 3 , only all the base sequences of shigella FUREKUSUNERI are shown, a dot shows the same base as the base sequence of shigella FUREKUSUNERI, and only the part of a different base is shown by the alphabet about the base sequence of other bacteria. Moreover, the part where a corresponding base does not exist is shown by the hyphen, and the array of the bacillus which has a base sequence to this field is indicated by the field in which the array of the gyrB gene of shigella FUREKUSUNERI does not exist. Drawing 4 thru/or drawing 11 to the response relation and the mismatch part of a base sequence of a total of 19 sorts of gyrB genes are quite obvious. That is, as for the base in said fragment, it is desirable [nine sorts of above-mentioned shigella groups or the above-mentioned nucleic acid for the Salmonella bacteria measurement] that it is what consists of the fragment which has a different base of the nucleic acid which has the base sequence shown in the array number 20 thru/or 29, respectively, or its complementary strand all over one of response fields at least. In addition, what is necessary is just the nucleic acid which only the bacillus measured as mentioned above is not limited to the nucleic acid containing the base from which all and the response base of these ten kinds of bacilli differ like the above, and these ten kinds of bacilli are desirable any one sort at least, and contains at least any bases [five sorts of] from which any seven sorts of response bases differ at least still more preferably.

[0023] This invention also offers two or more nucleic-acid chips for bacteria detection chosen from the group which consists of shigella FUREKUSUNERI which immobilizes two or more nucleic acid probes on a base material again among the nucleic acid probes for nine sorts of bacillus measurement of above-mentioned this invention, and changes, shigella BOIDI, shigella ZONEI, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium, Salmonella Chester, Salmonella enteritidis, and Salmonella ORANIEMBAGU. For such a nucleic-acid chip, it is preferably desirable among these nine kinds of nucleic acid probes for bacillus measurement to immobilize at least three or more kinds and the nucleic acid probe for detecting six or more kinds of all bacilli [these nine kinds of] most preferably. Ten kinds of above-mentioned bacilli with which the gyrB gene sequence is shown in the array number 20 thru/or 29 are desirable any one sort at least, and, as for the nucleic acid probe to immobilize, it is still more desirable that it is a nucleic acid containing the base from which any response bases [five sorts of / any seven sorts of] differ at least still more preferably at least. By doing so, it is because it also combines that the bacilli in a specimen are not the ten above-mentioned sorts of bacilli and it can be decided. In addition, it is possible to decide that the bacilli in a specimen are not the ten above-mentioned sorts of bacilli based on the pattern of whether to hybridize with two or more of other nucleic acid probes by using the combination of a nucleic acid probe in this case, even if it is the case where the nucleic acid probe which hybridizes two or more things of these ten sorts of bacilli is used as well as the case of discernment of nine sorts of above-mentioned shigella groups or the Salmonella bacillus. An example of the combination of such a nucleic acid probe is indicated by the following example 4.

[0024] When immobilizing the nucleic acid probe for two or more strain measurement and making it the above nucleic-acid chips, it is a target nucleic acid in a specimen (in this description). If it sets up so that each nucleic acid probe of this invention and the optimal conditions of the hybridization [nucleic acid / which should be detected / each] a "target nucleic acid" to hybridize may become the same substantially about all immobilization nucleic acid probes Since it can inspect good by contacting the whole nucleic-acid chip to magnification product liquid and coincidence, and making it hybridize on the same conditions, it is desirable. For this reason, as for the melting out temperature Tm of the double strand nucleic acid formed of the hybridization of the each nucleic acid probe and

target nucleic acid which immobilize for a nucleic-acid chip, it is desirable that less than 4 degrees C of differences of the highest thing and the minimum thing are less than 2 degrees C preferably. in order [moreover,] to raise the correctness of inspection -- the near center of each nucleic acid probe -- it is preferably desirable 3 / 10 - 7/10, and to have at least one mismatch part in the range of 4 / 10 - 6/10 still more preferably with the number of bases from one edge of a nucleic-acid fragment.
[0025] The nucleic-acid chip of this invention can be used like the conventional DNA chip. That is, the target nucleic acid in a specimen (namely, a gyrB gene or its part) is amplified by the nucleic-acid amplifying method of common knowledge like PCR using the primer of the couple which can amplify this target nucleic acid. The primer used at this time is not a species-specific primer, and it is desirable that it is what can amplify each target nucleic acid of all the bacillus origins that it is going to detect and/or identify. In addition, the bacillus which it is going to identify means the thing of the bacillus of other specific kinds in the case of deciding that the detected bacillus is not a bacillus of other specific kinds, and it is chosen from the nine above-mentioned sorts of shigella groups or the Salmonella bacillus and the ten above-mentioned sorts of Yersinia bacilli, the Enterobacter bacilli, the Vibrio bacilli, the Escherichia coli, and Salmonella paratyphi B other than the bacillus which should be detected here. By amplifying each target nucleic acid of all the bacillus origins that it is going to detect and/or identify, identification of a bacillus is attained with the electropositive pattern on a latter nucleic-acid chip. In addition, this can be easily performed based on the base sequence shown in drawing 4 thru/or drawing 11 that the combination of the primer which can amplify each target nucleic acid of all the bacillus origins that it is going to detect and/or identify should just set up the primer hybridized to two fields (however, one side complementary strand) to which all of the response field of all these bacilli are the same arrays. Or even if it is the primer hybridized to the field to which all of no response field of bacilli are the same arrays, each target nucleic acid of all the bacillus origins that it is going to detect and/or identify can be amplified also by using the mixed primer containing two or more complementary primers for each field which should be hybridized. In addition, it cannot be overemphasized that it is required for the field which each nucleic acid probe on a nucleic-acid chip hybridizes to exist in the field amplified. An indicator nucleotide is used as a nucleotide used as a raw material which constitutes a magnification nucleic acid in the case of magnification of a target nucleic acid. But an indicator nucleotide requires only one of four kinds of nucleotides, and three kinds of other nucleotides can use the usual nucleotide by which an indicator is not carried out. By using an indicator nucleotide, the target nucleic acid amplified is obtained as that by which the indicator was carried out. It can know whether each target nucleic acid about each nucleic acid probe was contained in the specimen by making this indicator magnification product hybridize with each nucleic acid probe on a nucleic-acid chip, and measuring the indicator combined on the base material after washing. And the bacillus contained [which / of two or more nucleic acid probes / hybridized and] in a specimen based on the pattern of the electropositive spot can be identified. In addition, all detection of the indicator combined on the above-mentioned actuation, i.e., magnification of a target nucleic acid, hybridization with a nucleic acid probe, and a base material etc. can be performed with a well-known conventional method.
[0026] Moreover, by this invention, since the base sequence of the gyrB gene of the nine above-mentioned sorts of close relationship bacteria was determined They are the nucleic acid which has the base sequence shown in the array numbers 1-9, or its fragment. At least by comparing with the base sequence shown in the array numbers 1-9 in it by amplifying the fragment which has other eight sorts of bases from which the base in this fragment differs all over one of response fields, and determining the base sequence of this fragment A bacillus can be identified (this approach may be hereafter called "direct sequencing method"). The field which will be made to identification of a seed by investigating the base sequence of the field as a field which should be amplified is desirable. Such fields may be all fields of the array shown in the array numbers 1-9, and may be fields narrower than it.
[0027] Since a base sequence is determined direct, in the case of the direct sequencing method, it is not necessary to use the primer which has the above mismatch parts, and the respectively complementary usual primer can be used for it to the ends of a magnification field.
[0028]
[Example] Hereafter, this invention is more concretely explained based on an example. But this

invention is not limited to the following example.
[0029] Decision of the base sequence of an example 1 gyrB gene (1) Biomass pretreatment shigella FUREKUSUNERI, shigella BOIDI, shigella ZONEI, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium, Salmonella Chester, Salmonella enteritidis, or Salmonella ORANIEMBAGU was semi- cultivated by 3ml of liquid culture with the conventional method, respectively. then, 12000 rpm -- centrifugal was carried out for 10 minutes and 4 degrees C of biomasses were collected. It dissolved in 50micro of purified water l, and this was heated 100 degrees C for 10 minutes. this -- 15000 rpm -- 4 degrees C, centrifugal was carried out for 10 minutes, supernatant liquid was moved to a new tube and this was made into DNA liquid.
[0030] (2) DNA of PCR ** was used as mold and PCR was performed. The base sequence of the used forward side primer UP1 and the reverse side primer UP2 is shown in the array numbers 10 and 11, respectively. a reaction presentation -- 10xPCR Buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15mM MgCl2, 0.001% (w/v) gelatin) every of 10microl and two mM(s) each -- dNTP 10microl and 50microM primer UP1 2microl and 50microM primer UP2 2micro -- l and AmpliTaq Gold (product made from PERKIN ELMER)0.5 It was made to react by mul and DNA 10microl. Conditions were made to react in 96-degree-C 1-minute and 60-degree-C 1 minute, and 72-degree-C 2 minutes and 60 cycle. The object product was checked by agarose gel electrophoresis 3%. About what has checked magnification, it refined using the G-50 sephadex column (Pharmacia manufacture). a purification object -- 3000 rpm -- centrifugal was carried out for 1 minute and it collected.
[0031] (3) Sequence was performed by the following reaction conditions using the sequence purification magnification product. The auto sequencer used ABI PRISM310 Genetic Analyzer (ABI company make). BigDye Terminator Cycle Sequencing and FS (ABI company make) were used for the reagent. The reaction condition followed the protocol of an ABI company.
[0032] Consequently, the base sequence shown in the array numbers 1-9, respectively was determined about nine sorts of above-mentioned bacilli.
[0033] Example 2 These three sorts of discernment was performed using the primer of this invention from identification shigella FUREKUSUNERI, shigella BOIDI, and shigella ZONEI of the bacillus using a primer, using as a specimen the DNA liquid prepared like the example 1.
[0034] As a forward side primer, six kinds of things as follows were used.
F-M1t: agcgtgacggcaaagaagatcatF-M1c: agcgtgacggcaaagaagaccacfl-Ma: agaacaaaacgccgatccacccafl-Mg: agaacaaaacgccgatccacccgbo-Mt: acaagaacaaaacgccgatccatbo-Mc: The R-125 following primer was used as acaagaacaaaacgccgatccac and a reverse side primer.
R-125: ctggaaaccatcgttccact [0035] The part which drew the underline is a mismatch part during each forward side primer array. In all, the three-dash terminal serves as a mismatch part at least. F-M1t and F-M1c is a 308nt - 330nt field, 327nt of F-M1t reaches, it sets 330nt to T as well as five sorts of Salmonella, and F-M1c sets these to C as well as three sorts of a shigella group. In addition, the array of F-M1c is completely [as the array of shigella FUREKUSUNERI and shigella BOIDI] the same, and since a mismatch part is not a three-dash terminal as shigella ZONEI is shown in the below-mentioned result, although 312nt differs, also in shigella ZONEI, magnification has broken out. It is a 377nt - 399nt field, and fl-Ma and fl-Mg of fl-Ma are completely [as the array of shigella FUREKUSUNERI] the same, and its fl-Mg is completely [as the array of shigella BOIDI and shigella ZONEI] the same. It is a 374nt - 396nt field, and bo-Mt and bo-Mc of bo-Mt are completely [as the array of shigella FUREKUSUNERI and shigella ZONEI] the same, and its bo-Mc is completely [as the array of shigella BOIDI] the same.
[0036] Six kinds of PCR was performed in each of the above-mentioned forward side [six kinds] primer, and the combination of the reverse side primer R-125. The reagent and the reaction condition were performed like the example 1.
[0037] A result is shown in the following table 1. In "O", magnification having broken out and "-" show that magnification did not break out among a table 1. Since the combination of the forward side primer in which magnification occurs differs for each kind as shown in a table 1, three sorts of these bacilli are discriminable by investigating by which forward side primer magnification broke out.
[0038]
[A table 1]

	F-Mlt	F-Mlc	fl-Ma	fl-Mg	bo-Mt	bo-Mc
S. flexneri	—	O	O	—	—	O
S. sonnei	—	O	—	O	—	O
S. boydii	—	O	—	O	O	—

[0039] Example 3 Identification of the bacillus using a probe (1) These six sorts of discernment was performed using the probe of this invention from specimen shigella FUREKUSUNERI, shigella BOIDI, shigella ZONEI, Salmonella typhi, Salmonella paratyphi A, and Salmonella enteritidis, using as a specimen the DNA liquid prepared like the example 1.
[0040] (2) The following nine kinds were used as a probe probe.
S1-CA tcggcatcg: cacccaaatS1-CG: cacccgaatS1-TG: catccgaatS2-C: ccaccgaaaS2-T: ccactgaaaS3-TGT: ttggcgttgS3-TGC: ttggcgtcgS3-CGG : tcggcgtggS3-CAC : [0041] Among these probes, S1-CA is completely [as the array of shigella FUREKUSUNERI] the same, it is a 394nt - 402nt field, and its S1-TG is [S1-CA S1-CG, and S1-TG of S1-CG are completely / as the array of shigella ZONEI, Salmonella typhi, and Salmonella enteritidis / the same, and] completely [as the array of shigella BOIDI and Salmonella paratyphi A] the same. It is a 416nt - 424nt field, and S2-C and S2-T of the array of S2-C are completely [as the array of shigella FUREKUSUNERI, shigella BOIDI, Salmonella typhi, and Salmonella enteritidis] the same, and its array of S2-T is completely [as the array of shigella ZONEI and Salmonella paratyphi A] the same. S3-TGT, S3-TGC, S3-CGG, and S3-CAC are 434nt - 442nt fields. The array of S3-TGT is completely [as the array of shigella FUREKUSUNERI] the same. The array of S3-TGC is completely [as the array of shigella BOIDI and shigella ZONEI] the same, is completely [as the array of shigella CHIFI] the same, and is completely [as the array of shigella PARACHIFI A] the same. [of the array of S3-CAC] [of the array of S3-CGG]
[0042] (3) It is preparation above-mentioned each probe of a membrane 100 After adjusting to pM/mu l, solid phase was carried out to the membrane every [50micro / l]. It irradiated by 1200x104microMJ/cm2 by UV cross linker, and the membrane for dot blots was prepared.
[0043] (4) As a radiolabel forward side primer of a sample, it is F-125. : The sample by which the radiolabel was carried out was prepared by performing PCR under radiolabel dCTP existence by using each specimen gene as mold, using the above R-125 as aaagcatttgttgaatat and a reverse side primer. PCR 10xPCR buffer5(100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM MgCl2, 0.001% (w/v) gelatin) microl, Ten mM d0.8 each (A, G, T) mul, [alpha-32P] dCTP(150microcurie)30microl, Every 50microM A primer F-125 and R-125 It was made to react by 1microl, AmpliTaq Gold0.3 (product made from PERKIN ELMER) microl, and DNA5microl. 25 cycles of conditions were made to react in 72-degree-C 2 minutes for 60-degree-C 1 minute for 96-degree-C 1 minute. G-sephadex 50 column refined the indicator object after the PCR indicator.
[0044] (5) The purification radiolabel object was added and was made to react to the membrane produced at hybridization and a development place 55 degrees C for 4 hours. The 2xSSC(s)-0.1% SDS washing solution was added and it washed 3 times for 10 minutes at the room temperature. Next, 0.1xSSC washed 50 degrees C for 10 minutes after 3 times washing by the 0.1xSSC(s)-0.1% SDS penetrant remover. The autoradiography cassette and the film for autoradiography (FUJIFILM) were set for this membrane in the dark room. - Autoradiography was performed at 80 degrees C overnight. Negatives were developed with the automatic developer (product made from KODAKKU).
[0045] A result is shown in the following table 2. It is shown among a table 2 that the sample combined "O" with the membrane, and having not combined "-" is shown. Since the combination of the probe to combine changes with each kinds as shown in a table 2, these six sorts are discriminable by the above-mentioned approach.
[0046]
[A table 2]

表2

	S1-CA	S1-CG	S1-TG	S2-C	S2-T	S3-TGT	S3-TGC	S3-CGG	S3-CAC
シゲラ・フレクスネリ	O	−	−	O	−	O	−	−	−
シゲラ・ゾネイ	−	O	−	−	O	−	O	−	−
シゲラ・ボイディ	−	−	O	O	−	−	O	−	−
サルモネラ・チフィ	−	O	−	O	−	−	−	O	−
サルモネラ・パラチフィA	−	−	O	−	O	−	−	−	O
サルモネラ・エンテリティディス	−	O	−	O	−	−	−	−	−

[0047] The same actuation as an example 1 was performed using each culture of decision Salmonella paratyphi B of the base sequence of an example of reference 1 gyrB gene, an Escherichia coli O-157 stock, Yersinia en TEROKORITIKA, Yersinia RUKERI, Enterobacter cloacae, Enterobacter aerogenes, Vibrio alginolyticus, a vibrion comp berry, vibrion diazo trophy dregs, and vibrion GAZOGENESU, and the base sequence of the gyrB field of these ten sorts of bacteria was determined. The determined base sequence is shown in the array numbers 20-29 of an array table, respectively. Moreover, that where the base sequence as which the gyrB gene of a total of 19 sorts of bacilli of these ten sorts of bacteria and the nine above-mentioned sorts of shigella groups, or the Salmonella bacteria was determined was aligned is shown in drawing 4 thru/or drawing 11 .
[0048] Example 4 Identification of the bacillus by the DNA chip (1) Chemosynthesis of 11 kinds of probes below production of a DNA chip was carried out.
Shi1 caagaacaaaacgcc (array number 30)
Shi21 attggcgttgaagtg (array number 31)
Shi22 cgatccatccgaata (array number 32)
Shi23 ttctccactgaaaaaga (array number 33)
Sal1 aacaagaataaaacgcc (array number 34)
Sal21 cggcgtcgaagta (array number 35)
Sal22 cggcgtggaagta (array number 36)
Sal23 tgaatatctcaacaagaat (array number 37)
Sal24 atcttctatttctccac (array number 38)
Esc1a acggtatcggcgt (array number 39)
[0049] The kind which each above-mentioned probe hybridizes, and its field (drawing 4 - 11 reference) are shown in the following table 3.
[0050]
[A table 3]

プローブ	ハイブリダイズする種	領域
Shi1	シゲラ・フレキシネリ シゲラ・ゾネイ シゲラ・ボイディ 大腸菌0157 エンテロバクター・クロアカエ エンテロバクター・アエロゲネス	376nt～390nt
Shi21	シゲラ・フレキシネリ	434nt～448nt
Shi22	シゲラ・ボイディ サルモネラ・パラチフィA	391nt～404nt
Shi23	シゲラ・ゾネイ サルモネラ・パラチフィA	413nt～429nt
Sal1	サルモネラ・チフィ サルモネラ・パラチフィA サルモネラ・パラチフィB サルモネラ・エンテリティデス サルモネラ・チェスター サルモネラ・オラニエンバーグ サルモネラ・チフィムリウム	374nt～390nt
Sal21	サルモネラ・パラチフィB サルモネラ・エンテリティデス	436nt～448nt
Sal22	サルモネラ・チフィ サルモネラ・チェスター サルモネラ・オラニエンバーグ サルモネラ・チフィムリウム	436nt～447nt
Sal23	サルモネラ・チフィ サルモネラ・パラチフィA サルモネラ・パラチフィB サルモネラ・オラニエンバーグ	364nt～382nt
Sal24	サルモネラ・チフィ サルモネラ・チフィムリウム エンテロバクター・アエロゲネス	404nt～420nt
Esc1a	サルモネラ・チフィ サルモネラ・パラチフィB サルモネラ・エンテリティディス サルモネラ・チェスター サルモネラ・オラニエンバーグ サルモネラ・チフィムリウム 大腸菌0157 エンテロバクター・アエロゲネス	430nt～441nt

[0051] The spotting solution after adjusting the above-mentioned probe to 200micro M/mu l (the trade name "Micro Spotting Solution" (Telechem International, product made from Inc) was prepared to equivalent ****, and last concentration M/mu [of 100micro] l.) The solid phase of this was carried out to slide glass with the commercial microarray production machine after pouring distributively on a microtiter plate. After making it dry one whole day and night, an SDS solution and distilled water washed 0.2%. Next, Na2BH4 solution washed, after heating with 95-degree C distilled water. Again, after an SDS solution and distilled water washed, it was made to dry and the DNA chip was produced.

[0052] (2) The DNA liquid prepared like the example 1 from preparation shigella flexible SHINERI, shigella BOIDI, shigella ZONEI, Salmonella enteritidis, Salmonella Chester, Salmonella ORANIEMBAGU, the Salmonella paratyphi A, Salmonella paratyphi B, the Salmonella typhi, Salmonella typhimurium, and Escherichia coli O-157 of a specimen was used as a specimen. Each DNA in each specimen was amplified by PCR using the primer F-137 of the following couple, and R-137. In addition, magnification fields are the 383nt - 519nt field of shigella flexible SHINERI, and a response field of each bacillus.
F-137: gaaggyggyatymargmrttR-137:tcytgraarcyrtcrttcca magnification was performed under radiolabel dCTP existence, and, thereby, the radiolabel of the magnification product was carried out. A reaction presentation 10xPCR buffer (100mM TrisHCl, pH 8.3, 500mM KCl, 15mM MgCl2, 0.001% (W/V) gelatin) 5microl, 2mM d (A, G, T, C) 10microl, radiolabel dCTP (trade name FluoroLink Cy5-dCTP) (1mM) 1microl, 50microM PrimerF-137 1microl, It was made to react by PrimerR-137 1microl, AmpliTaq Gold (DNA polymerase made from PERKIN ELMER) 0.3microl, and DNA 5microl. Conditions were made to react in 96-degree-C 1-minute and 60-degree-C 1 minute, and 72-degree-C 4 minutes and 60 cycle. G-sephadex 50 column (trade name) refined the indicator object after the PCR indicator.
[0053] (3) The purification Cy-5 indicator magnification product was added and was made to react to the DNA chip created at the hybridization point 40 degrees C for 4 to 18 hours. The 2xSSC-0.02% SDS washing solution was added and it washed once for 3 minutes at the room temperature. Next, it washed for 3 minutes and was made to dry by the 0.1xSSC penetrant remover. This was made to scan with a microarray readout machine, and data were analyzed.
[0054] (4) A result result is shown in drawing 12 and drawing 13 . In addition, in these drawings, the circle the probe name is indicated to be by the bottom shows the spot which immobilized the probe, it is the spot of a positivity [spot / which is smeared away black] (that is, it hybridized with the probe), and the thing of void shows a negative (that is, it did not hybridize with a probe) spot. As these drawings show, it turns out that the pattern of the spot used as a positivity changes with each bacilli, and a bacillus can be identified based on this pattern. In addition, as shown in a table 3, although it hybridizes with the gyrB gene fragment of two or more bacillus origins, as for all probes other than probe Shi21, by combining them shows that identification of a bacillus is possible based on the pattern of the spot used as a positivity.
[0055]
[Effect of the Invention] This invention enabled shigella FUREKUSUNERI, shigella BOIDI, shigella ZONEI, Salmonella typhi, Salmonella paratyphi A, Salmonella typhimurium, Salmonella Chester, Salmonella enteritidis, and Salmonella ORANIEMBAGU to identify by genetic screening and to measure promptly for the first time as above. Therefore, it is expected that this invention contributes to a diagnosis of the infectious disease caused with these bacilli or food poisoning, a therapy, and prevention dramatically.
[0056]
[Layout Table]
SEQUENCE LISTING <110> SRL, INC. <120> Nucleotides for Measuring Bacteria Belonging to Genus Shigella or Salmonera <130> 00660 <160> 39 [0057]
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